E.coli Curdlan Purification

Overview :

Curdlan molecules may have as many as 12,000 glucose units and are insoluble in water, alcohols and most organic solvents,while they are soluble in a diluted alkali solution (0.25 M NaOH).

We have two protocols for Curdlan purification from *E.coli* based on this property: curdlan is soluble in a NaOH solution.

Procedure n°1 :

Reference : Demonstration of Curdlan-type polysaccharide and some other in microorganisms with aniline blue, Itaru Nakanishi, Tokuya Harada and all, J. Gen. App!. Microbiol., 22,1-11(1976)

1) Mix the culture with an equal volume of 1 N NaOH and then centrifuge at 10,000 rpm for 10min to remove the cells.
2) Neutralize the resulting supernatant by adding 3 N HCl
3) A precipitate is formed. Collect it by centrifugation at 10,000rpm for 10min and wash three times with water by centrifugation. Then dehydrate with acetone and dry it in vacuo.
4) This precipitated polymer is the curdlan-type polysaccharide.

Procedure n°2 :

Reference : « Exopolymers from curdlan production: incorporation of glucose-related sugars by Agrobacterium sp. strain ATCC 31749. Jin W. Lee & all, Canadian Journal of Microbiology, 1997, Vol. 43, N° 2 : pages 149-156 »

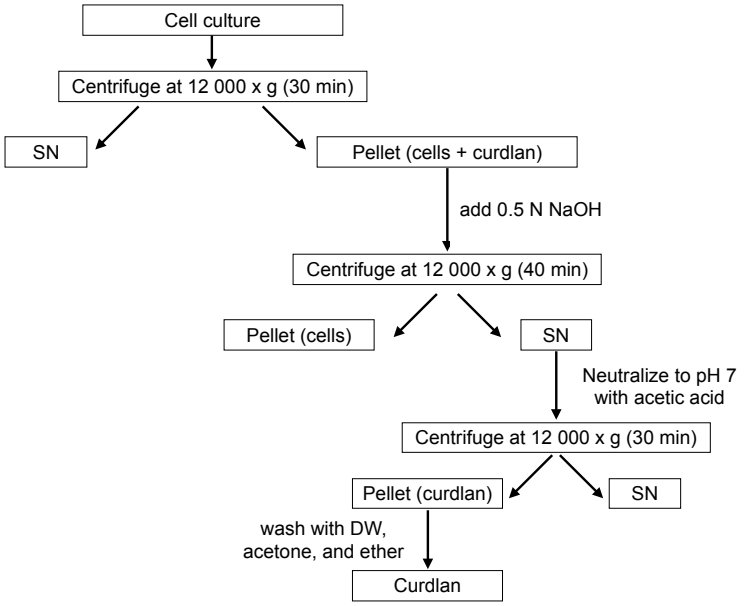

Fig. 1. Flow diagram depicting the purification procedure for curdlan. SN, supernatant.
Adapted from the reference.

1) Centrifuge the culture at 12000 x g for 30 min.
2) Add the pellet to an equivalent volume of 0.5 N sodium hydroxide at 3°C
3) Stir the mixture for 10 min and let it stand for 3 h at the same temperature.
4) Centrifuge the resulting viscous solution at 12000 x g for 40 min
5) Precipitate Curdlan in the clear supernatant by neutralization with 10% acetic acid
6) Repeatedly wash with DWater, acetone and ether.